

<b>9 BARBITURATE AND ACID DRUG QUANTITATION AND CONFIRMATION BY GC-NPD AND GCMS</b>	Page 1 of 5
<b>Division of Forensic Science</b>  <b>TOXICOLOGY TECHNICAL PROCEDURES MANUAL</b>	Amendment Designator:
	Effective Date: 31-March-2004
<p><b>9 BARBITURATE AND ACID DRUG QUANTITATION AND CONFIRMATION BY GC-NPD AND GCMS</b></p> <p><b>9.1 Summary</b></p> <p>9.1.1 Biological samples are made acidic with monosodium phosphate buffer (pH 5.1) and extracted with a mixture of n-butyl chloride and ethyl ether. The extract is derivatized with trimethylsulfonium hydroxide and an aliquot is injected into a GC equipped with an NPD detector for quantitation. The aliquot is subsequently confirmed using gas chromatography mass spectrometry.</p> <p><b>9.2 Specimen Requirements</b></p> <p>9.2.1 1 mL of fluid(s) or 1 g of tissue(s) or comparable amounts of fluid or tissue dilutions/homogenates</p> <p><b>9.3 Reagents and Standards</b></p> <p>9.3.1 Butalbital, 1 mg/mL</p> <p>9.3.2 Pentobarbital, 1 mg/mL</p> <p>9.3.3 Secobarbital, 1 mg/mL</p> <p>9.3.4 Phenobarbital, 1 mg/mL</p> <p>9.3.5 Amobarbital, 1 mg/mL</p> <p>9.3.6 Butabarbital, 1 mg/mL</p> <p>9.3.7 Glutethamide, 1 mg/mL</p> <p>9.3.8 Phenytoin (diphenylhydantoin), 1 mg/mL</p> <p>9.3.9 Carbamazepine, 1 mg/mL</p> <p>9.3.10 Cyclopentalbarbital (cyclopal), internal standard</p> <p>9.3.11 Trimethyl sulfonium iodide</p> <p>9.3.12 Monosodium phosphate</p> <p>9.3.13 Silver oxide</p> <p>9.3.14 Hexane</p> <p>9.3.15 Isoamyl alcohol</p> <p>9.3.16 Methanol</p> <p>9.3.17 Toluene</p> <p>9.3.18 N-butyl chloride</p> <p>9.3.19 Diethyl ether</p>	

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<div> <div>9.4 Solutions, Internal Standard, Calibrators, Controls</div> <div> <div>9.4.1 1.5 M monosodium phosphate buffer, pH 5.1 Add 103.4 grams of monosodium phosphate (NaH<sub>2</sub>PO<sub>4</sub>) to a 500 mL volumetric flask and QS to volume with dH<sub>2</sub>O.</div> <div>9.4.2 n-Butyl chloride:diethyl ether (95:5, v:v) Mix 950 mL n-butyl chloride with 50 mL diethyl ether .</div> <div>9.4.3 Toluene:Hexane:Isoamyl Alcohol (THIA) ( 78:20:2, v:v:v) Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol.</div> <div>9.4.4 Reconstituting solvent: Toluene/hexane/isoamyl alcohol/methanol, (59:15:1.5:25, v:v:v:v): Mix 25 mL methanol with 75 mL THIA</div> <div>9.4.5 Trimethyl sulfonium hydroxide derivatizing reagent Add 6.12 g trimethylsulfonium iodide, 7.39 g silver oxide, and 15 mL methanol to a 25 mL teflon capped test tube covered with aluminum foil (light sensitive reaction). Rotate for four or more hours, centrifuge, and decant the supernatant to an aluminum foil covered test tube. Keep refrigerated.</div> <div>9.4.6 Extraction Solvent containing Internal Standard: Weigh 20 mg of cyclopal free acid and transfer to a 10 mL volumetric flask. QS to volume with methanol for final concentration of 2 mg/mL). Aliquot 2 mL of 2 mg/mL cyclopentabarbital (cyclopal) stock solution into a 1000 mL volumetric flask and QS to volume with extraction solvent (n-butyl chloride:diethyl ether) to yield 4 mg/L cyclopal in extraction solvent.</div> <div>9.4.7 Drug stock solutions: <div> <div>9.4.7.1 If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free acid, transfer to a 10 mL volumetric flask and QS to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free acid, and weigh this amount.</div> </div> <div>9.4.8 Working Standard Solution A (0.1 mg/mL): Add 1.0 ml of each of the following 1 mg/mL stock solutions to a 10 mL volumetric flask: butalbital, phenobarbital, carbamazepine and phenytoin. QS to volume with methanol.</div> <div>9.4.9 Working Standard Solution B (0.1 mg/mL): Add 1.0 ml of each of the following stock solutions to a 10 mL volumetric flask: butabarbital, secobarbital, pentobarbital, and glutethamide. QS to volume with methanol.</div> <div>9.4.10 Blood calibrators, standards, and controls preparation: <div> <div>9.4.10.1 To prepare the following calibration curve, pipet the following volumes of working standard solution A into appropriately labeled 16 x 125 mm screw cap test tubes <div> <div>30 mg/L Calibrator300 µL of working standard solution A</div> <div>20 mg/L Calibrator200 µL of working standard solution A</div> <div>10 mg/L Calibrator100 µL of working standard solution A</div> <div>5 mg/L Calibrator50 µL of working standard solution A</div> <div>2 mg/L Calibrator20 µL of working standard solution A</div> <div>1 mg/L Calibrator10 µL of working standard solution A</div> </div> <div>9.4.10.1.1 Evaporate standards to dryness under nitrogen. Add 1 mL blank blood to each tube.</div> <div>9.4.10.2 Standard B contains rarely encountered drugs (butabarbital, secobarbital, pentobarbital, and glutethamide). During routine barbiturate analyses, run at least 1 standard containing working solution B for retention times. If any of the 4 drugs are present, a full calibration curve is required.</div> </div> </div> </div> </div></div></div>	

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9.4.10.2.1	For routine analyses, pipet 100µL of working Standard Solution B into a 16 x 125 mm labeled screw-cap test tube. Evaporate to dryness under nitrogen. Add 1 mL blank blood for a final concentration of 10mg/L.	
9.4.10.2.2	If a full calibration curve is required, pipet the following volumes of working standard solution B into appropriately labeled 16 x 125 mm screw cap test tubes	
	<ul style="list-style-type: none"> <li>30 mg/L Calibrator                      300 µL of working standard solution B</li> <li>20 mg/L Calibrator                      200 µL of working standard solution B</li> <li>10 mg/L Calibrator                      100 µL of working standard solution B</li> <li>5 mg/L Calibrator                        50 µL of working standard solution B</li> <li>2 mg/L Calibrator                        20 µL of working standard solution B</li> <li>1 mg/L Calibrator                        10 µL of working standard solution B</li> </ul>	
9.4.10.2.2.1	Evaporate standards to dryness under nitrogen. Add 1 mL blank blood to each tube.	
9.4.10.3	Controls	
9.4.10.3.1	Negative control. Blood bank blood (or comparable) determined not to contain barbiturates, phenytoin, carbamazepine and glutethamide.	
9.4.10.3.2	Positive control. In house control containing each analyte of interest from a different lot number or manufacturer than standards.	
<b>9.5</b>	<b>Apparatus</b>	
9.5.1	Agilent GC/MSD, Chemstation software, compatible computer & printer	
9.5.2	Agilent GC with Nitrogen-Phosphorous Detector, Chemstation software, compatible computer & printer	
9.5.3	Test tubes, 16 x 125 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps	
9.5.4	Test tubes, 16 x 114 mm (10 mL) glass, conical bottom	
9.5.5	Centrifuge capable of 2,000 – 3,000 rpm	
9.5.6	Vortex mixer	
9.5.7	Evaporator/concentrator	
9.5.8	GC autosampler vials and inserts	
9.5.9	Test tube rotator	
9.5.10	GC/NPD parameters. Instrument conditions may be changed to permit improved performance.	
9.5.10.1	Oven program.	
	<ul style="list-style-type: none"> <li>Equilibration time: 0.50 minutes</li> <li>Initial temp: 150° C</li> <li>Initial time: 0.5 minutes</li> <li>Ramp: 12° C/min</li> <li>Final Temp: 280° C</li> </ul>	

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<ul style="list-style-type: none"> <li>• Final Time: 4 minutes</li> <li>• Run Time: 15 minutes</li> </ul>		
9.5.10.2 Inlet.		
<ul style="list-style-type: none"> <li>• Mode: Splitless</li> <li>• Temperature: 250° C</li> <li>• Constant pressure: 16 psi</li> <li>• Purge flow: 49.6 mL/min</li> <li>• Total flow: 52.9 mL/min</li> <li>• Injection volume: 1.0 µL</li> </ul>		
9.5.10.3 Detector.		
<ul style="list-style-type: none"> <li>• Temperature: 290° C</li> <li>• Hydrogen flow: 3.0. mL/min</li> <li>• Air flow: 60 mL/min</li> <li>• Mode: Constant column + makeup flow</li> <li>• Combined flow: 20.0 mL/min</li> <li>• Injection volume: 1.0 µL</li> <li>• Makeup flow: On</li> </ul>		
9.5.10.4 Column: HP-5 25 m x 0.25 mm x 0.25 µm.		
9.5.11 GC/MSD parameters. Instrument conditions may be changed to permit improved performance.		
9.5.11.1 Acquisition Mode: Scan (50 – 550 amu)		
9.5.11.2 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm		
9.5.11.3 Detector Temperature: 280° C		
9.5.11.4 Basic drug screen. Instrument conditions may be changed to permit improved performance.		
9.5.11.4.1 Oven Program		
<ul style="list-style-type: none"> <li>• Equilibration time: 0.50 minutes</li> <li>• Initial temp: 110° C</li> <li>• Initial time: 1 minutes</li> <li>• Ramp: 10° C/min</li> <li>• Final Temp: 290° C</li> <li>• Final Time: 9 minutes</li> <li>• Run Time: 28 minutes</li> </ul>		
9.5.11.4.2 Inlet		
<ul style="list-style-type: none"> <li>• Mode: Splitless</li> <li>• Temperature: 270° C</li> <li>• Injection volume: 1.0 µL</li> <li>• Purge Time: ON at 1.0 minute</li> </ul>		

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<p><b>9.6 Procedure</b></p> <p>9.6.1 Label clean 16 x 125 mm screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs.</p> <p>9.6.2 Prepare calibrators and controls.</p> <p>9.6.3 Pipet 1 mL of each case sample into appropriately labeled tubes.</p> <p>9.6.4 Add 1 mL 1.5 M sodium phosphate buffer (pH 5.1) to each tube.</p> <p>9.6.5 Add 3 mL extraction solvent (n-butyl chloride/diethyl ether) containing cyclopal internal standard to each tube.</p> <p>9.6.6 Cap and rotate tubes for 30 minutes.</p> <p>9.6.7 Centrifuge at approx 2500 rpm for 10 minutes. Transfer organic (upper) layer to clean 5 mL conical bottom tubes. Discard lower layers.</p> <p>9.6.8 Add 50 µL methanol and 50 µL TMSH to each tube.</p> <p>9.6.9 Evaporate samples to dryness under nitrogen at 50-55° C. Note: Do not evaporate above 60° C.</p> <p>9.6.10 Reconstitute samples with 1.0 mL toluene/hexane/isoamyl alcohol/methanol reconstituting solvent. Vortex briefly.</p> <p>9.6.11 Transfer small aliquot to appropriately labeled GC vials and inject 1-2 µl on GC-NPD.</p> <p>9.6.12 Save remainder of reconstituted samples for confirmation by GC-MSD (if not already confirmed).</p> <p><b>9.7 Calculation</b></p> <p>9.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios versus calibrator concentration.</p> <p><b>9.8 Quality Control And Reporting</b></p> <p>9.8.1 See Toxicology Quality Guidelines</p> <p><b>9.9 References</b></p>	